Instruction Manual

FastDNA® Kit

One Call

Rapid Isolation of Genomic DNA from Plant and Animal Tissue, Bacteria, Yeast, Algae and Fungi Using the FastPrep® and FastPrep® 24 Instruments

One Source

Catalog # 6540-400
100 Preps

A World of Biotechnology Reagents

Storage:
Ambient temperature (15 – 30°C)

Revision # 6540-400-06APR

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1. Introduction to the FastDNA® Kit and the FastPrep® Instruments

The FastDNA® Kit quickly and efficiently isolates genomic DNA from a wide variety of sources. Designed for use with the FastPrep® Instruments from MP Biomedicals, plants, animal tissues, yeast, bacteria, algae, fungi and many other samples are easily lysed within 40 seconds. These benchtop devices use a unique, optimized motion to homogenize samples by multidirectional, simultaneous impaction with lysing matrix particles. FastPrep® Instruments provide an extremely quick, efficient and highly reproducible homogenization that surpasses traditional extraction methods using enzymatic digestion, sonication, blending, douncing and vortexing.

Samples are placed into 2.0 ml tubes containing Lysing Matrix A, irregularly shaped garnet particles and a single ¼ inch ceramic sphere. While almost all samples are easily processed with this pre-filled combination, additional ¼ inch ceramic spheres are provided for hard samples such as bone, cartilage or seeds.

Homogenization in the FastPrep® Instrument with Lysing Matrix A takes place in the presence of sample-specific Cell Lysis Solutions (CLS). For plant tissues, CLS-VF is used in conjunction with a Protein Precipitation Solution (PPS). Yeast, algae and fungi are lysed in the presence of CLS-Y. For all other samples, CLS-TC is used during sample lysis. For maximum flexibility, all buffers are provided in the kit.

Following lysis, samples are centrifuged to pellet debris and lysing matrix. DNA is purified from the supernatant with a silica-based GENECLEAN® procedure. Eluted DNA is ready for digestion, electrophoresis, PCR and any other desired application.

2. Kit Components and User Supplied Materials

2.1 FastDNA® Kit Components

Lysing Matrix A	100x 2.0 ml tubes
¼ Ceramic Spheres	100 spheres
Binding Matrix	66 ml
Concentrated SEWS-M	12 ml
DES	25 ml
CLS-VF	90 ml
PPS	25 ml
CLS-TC	110 ml
CLS-Y	110 ml
User manual	1 each
MSDS	1 each
Certificate of Analysis	1 each

2.2 User Supplied Materials

FastPrep® Instrument (see Section 8)
Microcentrifuge that can freely spin 2.0 ml tubes
Microcentrifuge tubes (2.0 ml and 1.5 ml)
Rotator or low-speed vortex
(Optional) SPIN Filters and Catch Tubes, 100
(Cat # 2080-800)

3. Important Considerations Before Use

3.1 Preparation of SEWS-M Wash Solution

The FastDNA® Kit contains a bottle with 12 ml of Concentrated SEWS-M Wash Solution. Before using this solution, add 100 ml of 100% ethanol and mark on the bottle label the date ethanol was added. Ensure that the bottle is securely closed to prevent evaporation, and store at room temperature.

3.2 Precipitation in CLS-TC Buffer

If the FastDNA® Kit was shipped or stored at a low temperature, a harmless precipitate may form in the CLS-TC Buffer. If a precipitate is seen, incubate the bottle in a 45-55°C water bath for several minutes and mix to bring the precipitate back into solution. Allow solution to cool to room temperature.

3.3 Sample Lysis with the FastPrep® Instrument

The fill volume in the lysing matrix tube after the addition of the Cell Lysis Solution to the sample should allow sufficient air space in the sample tube for efficient FastPrep® Instrument processing. MP Biomedicals recommends using 100 – 200 mg of starting material as long as there is between 250 – 500 µl of empty space in the tube. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

MP Biomedicals' Lysing Matrix particles and tubes have been rigorously tested and validated in the FastPrep® Instruments. The use of other products with the FastPrep® Instruments is not recommended and may result in sample loss or instrument failure.

A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse almost all samples. If the user experimentally determines that additional processing time is required, the sample should be incubated on ice in the Lysing Matrix A tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent overheating the sample and tube.

MP Biomedicals recommends that all researchers begin the protocol with the Lysing Matrix A as supplied in the kit (garnet matrix and single sphere). If lysis is inefficient even after multiple runs of 40 seconds, an additional ¼ inch ceramic sphere (provided) can be added on top of the sample. Depending on the sample, lysis and/or yield may or may not improve and shearing of existing genomic DNA may begin to occur. Samples with 2 spheres should be processed carefully in order to balance increased yield and lysis against increased DNA shearing by varying speed and/or time settings

3.4 Recovery of DNA from Dry Samples

To optimize DNA recovery from extremely dry samples, leave the lysed sample at room-temperature in the Lysing Matrix A tube for an incubation period of 15 minutes to 2 hours after processing in the FastPrep® Instrument.

3.5 Co-Purification of RNA

Some tissues (i.e. liver, kidney) contain very high levels of RNA which may co-purify with the genomic DNA. If absolute control of RNA contamination is necessary, the final eluted DNA can be treated with RNase as per the manufacturer's protocol.

3.6 Use of optional SPIN filters

MP Biomedicals strongly recommends the use of SPIN filters during the silica-based GENECLEAN® procedure for final purification of genomic DNA. Although the traditional bind-wash-elute procedure is fine for almost all standard applications, the use of SPIN filters eliminates accidental transfer of silica particles and can also lead to increased purity of the final sample. Spin filters and catch tubes can be purchased separately (Cat # 2080-800), or a FastDNA SPIN Kit can be purchased (Cat # 6540-600).

4. Safety Precautions

Binding Matrix contains components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucus membranes (gloves, lab coat, and eye protection). Consult the enclosed Material Safety Data Sheet for additional details.

5. Protocol

1. Add sample to Lysing Matrix A tube. Place up to 100 - 200 mg tissue (fresh, frozen, dried etc.), or 200 µl of cells suspended in water or isotonic saline solution. For bacteria, yeast, algae, or tissue culture cells grown in suspension: Centrifuge a sufficient volume of culture to provide a pellet size of 50-00 mg wet weight or up to 10^9 bacteria, 10^8 yeast/algae, or 10^7 mammalian cells. Resuspend pellets in water or isotonic saline to give a maximum suspension volume of 200 µl.

   NOTE: See section 3.3 for other important guidelines.

2. Add appropriate Cell Lysis Solution (CLS) according to table below:

Processing Tissue From:	**Add to Sample Tube:**
Plant tissue	800 µl CLS-VF and 200 µl PPS
Animal tissue, cultured cells, insects, bacteria, bone, etc.	1.0 ml CLS-TC
Yeast, algae or fungi	1.0 ml CLS-Y

3. Homogenize in the FastPrep® Instrument for 40 seconds at a speed setting of 6.0.

4. Centrifuge at 14,000 x g for 5-10 minutes to pellet debris.

5. Transfer supernatant (700–800 µl) to a 2.0 ml microcentrifuge tube and add an equal volume of Binding Matrix. Invert to mix.

   NOTE: It is important to use a tube that is large enough to allow room for complete mixing of the entire volume during the course of the next step. Tubes with conical bottoms are not recommended. A 2.0 ml microcentrifuge tube works well at this step.

6. Incubate with gentle agitation for 5 minutes at room temperature on a rotator.

   NOTE: A low-speed vortex may be used at this point, but care must be taken not to shear the DNA.

7. Centrifuge at 14,000 x g for 1 minute to pellet the Binding Matrix. Discard the supernatant.

8. Add 500 µl prepared SEWS-M and gently resuspend the pellet using the force of the liquid from the pipet tip.

NOTE: Ensure that ethanol has been added to the Concentrated SEWS-M. See section 3.1.

9. Centrifuge at 14,000 x g for 1 minute and discard the supernatant.

   NOTE: If using a SPIN filter, transfer resuspended Binding Matrix to a SPIN Filter. Centrifuge at 14,000 x g for 1 minute and discard content of the Catch Tube.

10. Centrifuge a second time at 14,000 x g for 1 minute and remove residual liquid with a small pipet tip.

    NOTE: If using a SPIN Filter, centrifuge a second time at 14,000 x g for 1 minute and replace the Catch Tube with a new, clean tube.

11. Elute DNA by gently resuspending Binding Matrix in 100 µl of DES. Incubate for 5 minutes at 55˚C in a heat block or water bath.

12. Centrifuge at 14,000 x g for 1 minute. Transfer eluted DNA to the clean microcentrifuge tube. DNA is now ready for downstream applications. Store at -20°C for extended periods or 4°C until use.

    NOTE: Avoid transferring particles of Binding Matrix with eluted DNA. To avoid shearing, transfer the DNA carefully using a pipet tip.

    NOTE: If using a SPIN Filter, centrifuge at 14,000 x g for 1 minute to bring eluted DNA into the clean catch tube. DNA is now ready for downstream applications. Store at -20°C for extended periods or 4°C until use.

6. Recommended Reference Format for Publications

DNA was isolated from (specific sample) using the FastDNA® Kit and the FastPrep® Instrument (MP Biomedicals, Irvine CA).

7. References

A wide variety of references for lysis and purification with FastPrep® products can be found on the Qbiogene website at http://www.qbiogene.com/fastprep.

8.Related Products

Description	Size	Catalog #
FastPrep® 24 Instrument	100-230V	6002-500
FastPrep® FP100A Instrument	100V	6001-100
FastPrep® FP120A Instrument	120V	6001-120
FastPrep® FP220A Instrument	220V	6001-220
FastDNA® SPIN Kit	100 preps	6540-600
FastDNA® SPIN Kit for Soil	50 preps	6560-200
FastRNA® Pro Soil-Direct Kit	50 preps	6070-050
FastRNA® Pro Soil-Indirect Kit	50 preps	6075-050
FastRNA® Pro Red Kit (Yeast & Fungus)	50 preps	6035-050
FastRNA® Pro Green Kit (Plant & Animal)	50 preps	6045-050
FastRNA® Pro Blue Kit (Bacteria)	50 preps	6025-050
FastProtein™ Blue Matrix	50 preps	6550-400
FastProtein™ Red Matrix	50 preps	6550-600
Lysing Matrix A	50 x 2 ml tubes	6910-050
Lysing Matrix A	100 x 2 ml tubes	6910-100
Lysing Matrix A	500 x 2 ml tubes	6910-500
SPIN Modules	100 Filters and Catch Tubes	2080-800

9. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices in order to diagnose, cure, mitigate, treat or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and the sole liability of MP Biomedicals hereunder shall be limited to, at our discretion, no replacement or compensation, product credits, refund of the purchase price of, or the replacement of materials that do not meet our specification. By acceptance of the product, Buyer indemnifies and holds MP Biomedicals harmless against, and assumes all liability for, the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refund or replacement is conditioned on Buyer notifying Qbiogene, Inc. within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

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